Attorney's Docket No.: 00786-625006/ MGH-1006.3 Torchilin CON

RECEIVED
CENTRAL FAX CENTER

MAR 3 0 2005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Vladimir P. Torchilin *et al.*
Serial No. : 10/081,223
Filed : February 22, 2002
Title : NUCLEOSOME-BASED ANTI-TUMOR COMPOSITIONS

Art Unit : 1642
Examiner : Susan Ungar, Ph.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. VLADIMIR P. TORCHILIN

I, Dr. Vladimir P. Torchilin, declare as follows.

1. I am an inventor of the subject matter claimed in the patent application referenced above. I am also a Professor in, and the Chairman of, the Department of Pharmaceutical Sciences of Northeastern University in Boston, Massachusetts.

2. The data presented here were obtained from studies that I conducted or that were conducted under my supervision.

3. The data presented here indicate that we can inhibit malignant cell growth in mammals at risk for such growth by administering nucleosomes. Our data further indicate that we can inhibit malignant cell growth even if anti-nuclear autoantibodies (ANAs) are already present in the mammals when the nucleosomes are administered.

4. We tested the ability of nucleosomes to inhibit tumor growth by administering nucleosomes to nude mice and then administering tumor cells from one of two tumor cell lines: NCI-H82 or PC-3. We prepared the nucleosomes from minced, homogenized rat livers using a standard procedure (Wassarman and Wolffe, *Methods in Enzymology*. 304:314-317, 1995), and we administered 50 μg of nucleosomes in 100 μl of saline to each mouse by subcutaneous injection. The nucleosomes were administered three times, once every two weeks. Between the second and third immunizations, we subcutaneously injected approximately 10 million tumor cells into the left flank of each animal in the treatment group ("the nucleosome-treated animals").

5. We measured the levels of ANAs in the nucleosome-treated animals approximately every five days, beginning on the day the animals received the first dose of nucleosomes. We also measured the levels of ANAs in the blood of comparable mice that were injected with tumor cells but not with nucleosomes ("the control animals"). ANAs were present in the circulation of

both the nucleosome-treated animals and the control animals. At the initial measurement, the ANA titre in the nucleosome-treated animals and the control animals was essentially the same. Over time, the ANA titre in the nucleosome-treated animals increased about five-fold. ANAs remained detectable in control animals at about, or somewhat above, their initial level. These results are presented graphically in Figure 1. Along the time line of the X-axis, the three filled arrows mark the administration of nucleosomes to the nucleosome-treated animals and the open arrow marks the administration of tumor cells to both the nucleosome-treated animals and the control animals. ANA levels in the nucleosome-treated animals are represented by dark squares (upper line) and ANA levels in the control animals are represented by lighter diamonds (lower line).

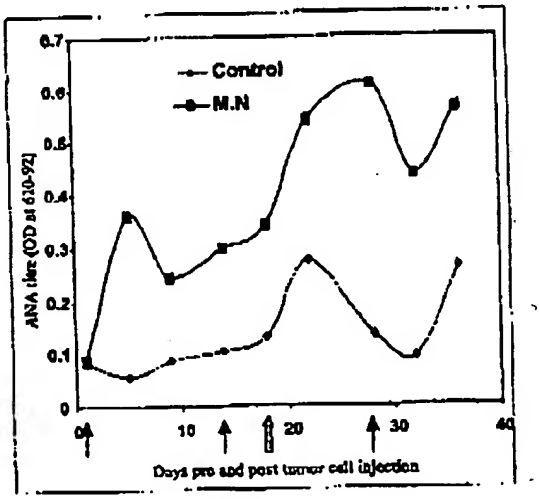

Figure 1

6. We measured the volume of the tumor (in mm^3) at various times after the tumor cells were injected and, after sacrificing the animals, we removed the tumors and weighed them (in g). The average volume and average weight of the tumors in the nucleosome-treated animals was consistently less than the average volume and average weight of the tumors in the control animals. The results were similar regardless of whether the tumor grew from NCI-H82 tumor cells (see Figure 2) or PC-3 tumor cells (see Figure 3). In each case, the lighter lines and bars

represent the results we obtained with the control animals, and the darker lines and bars represent the results we obtained with the nucleosome-treated animals.

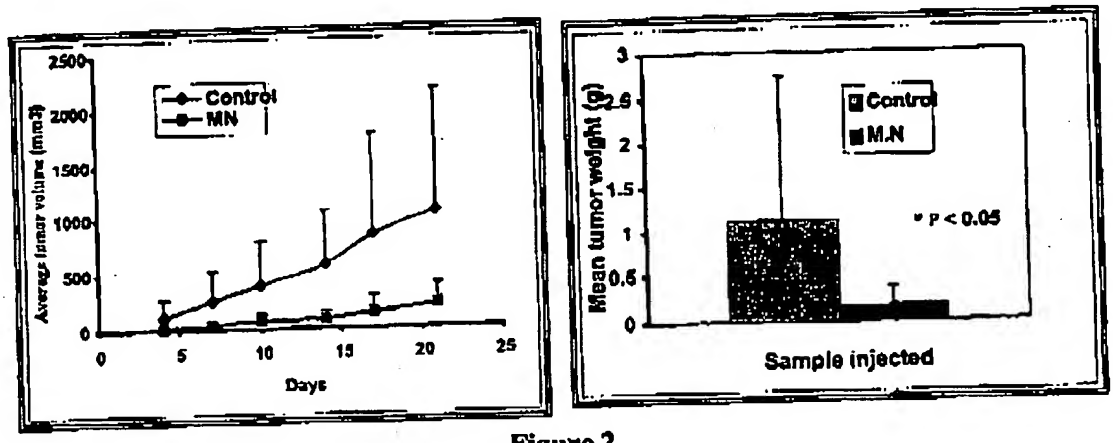

Figure 2

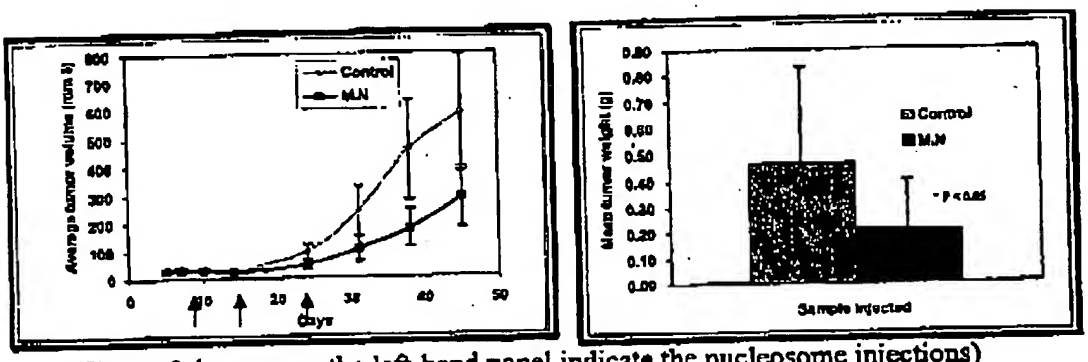

Figure 3 (arrows on the left-hand panel indicate the nucleosome injections)

7. A parametric t-test for two independent samples was used to estimate the significance of differences between the results we obtained with the nucleosome-treated animals and the results we obtained with the control animals.

8. Based on the results we obtained in a well-accepted animal model of tumor prophylaxis, I concluded that one could inhibit malignant cell growth in a mammal at risk for such growth by administration of nucleosomes. ANAs were present in both the nucleosome-treated animals and the control animals at the beginning of the study. When those levels were boosted by administration of nucleosomes, malignant cell growth was inhibited. When those levels remained at or around their natural baseline, the animals (here, the control animals) were

not protected from malignant cell growth (or were not protected nearly as well as the nucleosome-treated animals).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Dated: 3-21-05

Vladimir P. Torchilin, Ph.D.

21013107.doc